

Application No.: 10/081,555

Attorney Docket No.: SALK2270-5

Filing Date: February 20, 2002

(088802-5212)

Amendment in Response to Office Action (mailed 11/19/04) faxed 12/17/04

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Amendments to the Claims/Listing of Claims

Please amend claims 2 and 27-29, cancel claims 1, 6-26, 30 and 31, and add claims 32-37 as follows. This listing of claims will replace all prior versions, and listings of claims in the application.

1. (Cancelled)

2. (Withdrawn-currently amended) The method according to claim [[1]] 4, wherein said SXR polypeptide is a member of the steroid/thyroid hormone superfamily and forms a heterodimer with retinoid X receptor, wherein said heterodimer binds to a direct or inverted repeat response element comprising at least two half sites RGBNNM separated by a spacer of 0 up to 15 nucleotides,

wherein:

R is selected from A or G;

B is selected from G, C, or T;

each N is independently selected from A, T, C, or G; and

M is selected from A or C;

with the proviso that at least 4 nucleotides of said RGBNNM sequence are identical with the nucleotides at corresponding positions of the sequence AGTTCA; and wherein said SXR polypeptide activates transcription of gene(s) under the control of cytochrome P450 response element in response to a wide variety of natural and synthetic steroid hormones, compounds that induce catabolic enzymes, steroid receptor agonists and antagonists, and bioactive dietary compounds.

3. (Withdrawn) A method for screening a collection of compounds to determine those compounds which bind to a SXR polypeptide, or functional fragments thereof, said method comprising employing an SXR polypeptide in a binding assay.

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4. (Original) A method of testing a compound for its ability to regulate transcription-activating effects of a SXR polypeptide, said method comprising assaying for the presence or absence of reporter protein upon contacting a host cell containing said SXR polypeptide and a reporter vector with said compound, wherein said reporter vector comprises:

- (a) a promoter that is operable in said host cell,
- (b) a response element, and
- (c) DNA encoding a reporter protein,

wherein said DNA is operatively linked to said promoter for transcription of said DNA, and,

wherein said promoter is operatively linked to said SXR response element for activation thereof.

5. (Withdrawn) A method of identifying compounds which activate SXR polypeptide, but do not activate other members of the steroid/thyroid hormone superfamily, said method comprising:

(i) detecting in a first assay system the presence or absence of a first reporter protein upon contacting a first host cell with test compound(s), wherein said first host cell contains said SXR polypeptide and a first reporter vector, wherein said first reporter vector comprises:

- (a) a first promoter that is operable in said first host cell,
- (b) a SXR response element, and
- (c) a first DNA encoding a first reporter protein,

wherein said first DNA is operatively linked to said first promoter for transcription of said first DNA, and,

wherein said first promoter is operatively linked to said SXR response element for activation thereof;

(ii) detecting in a second assay system the presence or absence of a second reporter protein upon contacting a second host cell with test compound(s), wherein said second host cell contains a member of the steroid/thyroid hormone superfamily other than SXR and a second reporter vector, wherein said second reporter vector comprises:

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- (a) a second promoter that is operable in said second host cell,
- (b) a response element for said member of the steroid/thyroid hormone superfamily other than SXR, and
- (c) a second DNA encoding a second reporter protein,

wherein said second DNA is operatively linked to said second promoter for transcription of said second DNA, and

wherein said second promoter is operatively linked to said response element for said member of the steroid/thyroid hormone superfamily other than SXR for activation thereof; and

(iii) identifying those compounds which induce production of a reporter protein in said first assay, but not in said second assay, as compounds which activate SXR polypeptide, but do not activate other members of the steroid/thyroid hormone superfamily.

6-26. (Cancelled)

27. (Withdrawn-currently amended) Cells The method of claim 4 wherein said host cells comprise cells transfected with an isolated or recombinant polynucleotide, wherein said polynucleotide encodes a SXR polypeptide, or functional fragments thereof,

wherein said SXR polypeptide is a member of the steroid/thyroid hormone superfamily and forms a heterodimer with retinoid X receptor,

wherein said heterodimer binds to a direct or inverted repeat response element comprising at least two half sites RGBNNM separated by a spacer of 0 up to 15 nucleotides,

wherein:

R is selected from A or G;

B is selected from G, C, or T;

each N is independently selected from A, T, C, or G; and

M is selected from A or C;

with the proviso that at least 4 nucleotides of said RGBNNM sequence are identical with the nucleotides at corresponding positions of the sequence AGTTCA; and

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wherein said SXR polypeptide activates transcription of gene(s) under the control of cytochrome P450 response element in response to a wide variety of natural and synthetic steroid hormones, compounds that induce catabolic enzymes, steroid receptor agonists and antagonists, and bioactive dietary compounds.

28. (Withdrawn-currently amended) **Cells according to The method of claim 27**  
wherein said functional fragments of said SXR polypeptide comprise a ligand binding domain, a DNA binding domain or both.

29. (Withdrawn-currently amended) **Cells according to The method of claim 27**  
wherein said host cells are further transfected with a vector which comprises:

- (a) a promoter that is operable in said cells;
- (b) a response element, and
- (c) DNA encoding a reporter protein,

wherein said DNA is operatively linked to said promoter for transcription of said DNA,  
and

wherein said promoter is operatively linked to said response element for activation  
thereof.

30-31. (Cancelled)

32. (New) The method of claim 4 wherein said promoter is selected from the group consisting of an albumin promoter/enhancer, a CYP3A cellular promoter, a CYP3A23 cellular promoter, a CYP3A2 promoter, a CYP3A3 promoter, a CYP3A4 cellular promoter, and a 3A6 promoter.

33. (New) The method of claim 4 wherein said response element is a direct or inverted repeat response element comprising at least two half sites RGBNNM separated by a spacer of 0 up to 15 nucleotides,

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wherein:

R is selected from A or G;

B is selected from G, C, or T;

each N is independently selected from A, T, C, or G; and

M is selected from A or C;

with the proviso that at least 4 nucleotides of said RGBNNM sequence are identical with the nucleotides at corresponding positions of the sequence AGTTCA.

34. (New) The method of claim 33 wherein said response element is selected from the group consisting of an inverted repeat separated by a 6 nucleotide spacer (IR-6), a direct repeat separated by a 3 nucleotide spacer (DR-3), a direct repeat separated by a 4 nucleotide spacer (DR-4), and a direct repeat separated by a 5 nucleotide spacer (DR-5).

35. (New) The method of claim 34 wherein said response element is selected from the group consisting of:

AGGTCAN<sub>n</sub>AGGTCA,

wherein n is 3, 4, or 5 (SEQ ID NOS: 15, 16 and 17);

AGTTCAN<sub>n</sub>TGAACCT,

wherein n is 3, 4 or 5 (SEQ ID NO: 22); and

TGAACTN<sub>n</sub>AGGTCA), wherein n is 6 (SEQ ID NO:23).

36. (New) The method of claim 35 wherein said response element is selected from the group consisting of :

tagac AGTTCA tga AGTTCA tctac (SEQ ID NO:3),

taagc AGTTCA taa AGTTCA tctac (SEQ ID NO:4),

actgt AGTTCA taa AGTTCA catgg (SEQ ID NO:5),

caatc AGTTCA acag GGTTCA ccaat (SEQ ID NO:6),

cac AGGTGA gctg AGGCCA gcagc AGGTGCG aaa (SEQ ID NO:7),

gtgca GGTTCA actgg AGGTCA acatg (SEQ ID NO:8),

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gtgct GGTTCA actgg AGGTCA gtatg (SEQ ID NO:9),  
agtct AGTTCA gtggg GGTTCA gcctt (SEQ ID NO:10), and  
gagat GGTTCA agcaa GGGTCA ttaac (SEQ ID NO:11).

37. (New) The method of claim 4 wherein said reporter protein is luciferase or  $\beta$ -galactosidase.

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